بسم الله الرحمن الرحيم

Effect of *Boscia Senegalensis* leaves Water Extract on blood contents of rats

By:

Assmaa Ata Gobarah Mohammed Ali

B.Sc 2003 University of Khartoum

Faculty of Veterinary Medicine

Athesis Submitted for partial Ful Fillment of

The Master Degree in Biochemistry

Supervisor

Prof: Omer Fadul Idris

Faculty of Science &Technology

Alneelain University

Faculty of Veterinary Medicine

Khartoum University

May 2010

DEDICATION

To my lovely parents

To my child and my husband

To my sisters and brothers

To my friends

To all those whom I love

Assmaa

Acknowledgements

I am extremely grateful to my supervisor Prof Omer Fadul Idris for his excellent and wise guidance and continuous encouragement during the study period.

I am grateful to staff members of Medicinal and Aromatic Plants Research Institute (MAPRI).

I extend my thanks to the staff members of Laboratory Center in Khartoum hospital especially Susan Mustafa for assistance in analysis of samples.

I am highly acknowledging the technical assistance of Mohammed Aldo who contributed to this work.

Great acknowledgement is extended to my family, friends, teachers and any one donated help and even hopes.

List of Contents

Page

Chapter Three:The Results

List of Tables

List of Figures

List of abbreviations

ALT	Alanine amino transferase
AST	Aspartate amino transferase
ALP	Alkaline phosphatase
dl	Deciliter
g	Gram
Kg	Kilogram
L	Liter
mg	Milligram
mmol	Millimole
NADH	Nicotinamide adenine dinuclutide (reduced)
NAD^+	Nicotinamide adenine dinuclutide (oxidized)
SE	Standard error
Hb	Hemoglobin
RBCs	Red Blood Cells
WBCs	White Blood Cell
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
H& E	Hematoxylin and Eosin

Abstract

The objective of this study was to investigate the effect of ***Boscia senegalensis*** leaves water extract on blood contents (RBCs ,WBCs ,Hb, MCV,MCH,MCHC, and platelets count) and on serum ALT,AST, ALP, , Urea, Creatinine, amino acids composition , sodium and potassium on Wister albino rats.

The water extract of dry powder of ***B. senegalensis*** leaves was prepared using 250 g from the plant with 1500 ml and dried by Freez drier apparatus Trivac, USA .

Twenty four healthy male Wister albino rats were used in this experiment. They were divided into three groups eight rats in each on the basis of body weight ,the average weight of rats in each group was 75g.Group A kept as Control ,group B treated by ***Boscia senegalensis*** leaves water extract (200g/100g Bwt),Group C treated by ***Boscia senegalensis*** leaves water extracts (400g/100g Bwt) .

Blood samples were collected after one, two and three weeks respectivly and used to estimate some biochemical parameters in plasma. This study showed a significant effect on Hb and RBCs .There was an increase on Hb and decreased RBCs for two group (B and C) compared with control. Also this study showed no significantly changes on serum level of ALT, AST ,ALP, Creatinine, Urea and serum electrolytes. From all previous findings the result is ***Boscia senegalensis*** leaves water extract on dose 400mg/kg has toxic effect on Wister albino rats.

المستخلص

تم في هذه التجربة دراسة اثر المستخلص المائي لأوراق نبات المخيط علي محتويات الدم(كريات الدم الحمراءوالبيضاء وتركيزالهيموغلوبين ومتوسط حجم الكرية الحمراءومتوسط الهيموغلوبين الكلي للكريات ومتوسط الهيموغلوبين للكريه وعدد الصفائح الدموية) ومستوي (انزيمات الالنين امينو ترانسفيريز, الاسبرتيت امينو ترانسفيريز والالكلاين فوسفتيز)واليوريا والكرياتنين والاحماض الامينية,صوديوم وبوتاسيوم فى سيرم ذكور جرزان وسترالبيضاء.حضر المستخلص المائي لاوراق المخيط باستخدام 250جم من بودرة النبات مع لترونص ماء دافئ وجفف بالتبريدالجاف بواسطة جهازفريز دريير. اجريت التجربة في هذا البحث علي24 من ذكور جرزان وسترالبيضاءوالتى قسمت الى ثلاثة مجموعات تحتوي كل مجموعة علي ثمانيةجرذان بمتوسط وزن حوالي 75جم لكل مجموعة. المجموعة الاولي مجموعة ضبط ومراقبة للمجموعات الاخري, والمجموعات الثانية جرعت بمستخلص االمائى 200 مل جرام لكل كيلوجرام من وزن الجرذان., الثالثة جرعت بمستخلص المائى 400 مل جرام لكل كيلوجرام من وزن الجرذان.

بعد أسبوع ,أسبوعين, ثلاثة أسابيع من بداية التجربة على التوالي أخذت عينات من دم الجرذان ولوحظ بعد التحليل المعملي المستخلص المائي لأوراق نبات المخيط احدث تغييرمعنوى فى محتويات الدم وذلك بنقص في عدد كريات الدم الحمراء وزيادة فى تركيز الهيموغلوبين فى المجموعتين الثانية والثالثة مقارنة بمجموعة الضبط.لوحظ ايضا أن المستخلص المائي لأوراق نبات المخيط لم يتسبب في تغيير معنوي في مستوي إنزيمات(الالنين امينو ترانسفيريز, الاسبرتيت امينو ترانسفيريز والالكلاين فوسفتيز), الكرياتنين, اليوريا,الأحماض الامينية والالكتروليت في السيرم . مما سبق نستخلص أن المستخلص المائي لأوراق نبات المخيط بجرعة 400 مل جرام لكل كيلوجرام لها تأثير سام في جرزان وستر البيضاء.

Introduction

Boscia senegalensis or Aizen, is a member of the family Capparaceae. The plant originated from West Africa. Still a traditional food plant in Africa, this little-known fruit has potential to improve nutrition, boost food security, foster rural development and support sustainable land care **(NRC , 2008)**.

Synonyms are *Porrdia Senegalensis* (pers)-and *Boscia octandra* (Hochste Rodlk) The local name in Sudan ,Mukheit Umnkeit and krusan and widely spread in North kordofan and Darfour **(ELgazali *etal* .,1987).**

Mukheit or Aizens are not fruit crops in normal sense. It is the combination of foods and useful qualities that makes them important. The species produces enough different products to sustain human life almost by itself. In at least a dozen countries, at times people virtually live off Aizen fruits, Aizen seeds, Aizen leaves and berries are commercially available in many parts northern Nigeria and Sudan as good condiment for soups in times of scarcity **(NRC , 2008).**

This plant has a lot of uses, the leaves are traditionally used in west Africa in cereal protection against pathogens, Pharmacologic application and food processing **(Dicko *etal*,2001).**

In Sudan whole plant use as anthelimentic were as the emulsion of leaves used as an eye wash **(ELgazali *etal* ., 1997).**

The objectives :

Because of different uses of *Boscia senegalensis,* this study was aimed to evaluate the effect of *Boscia senegalensis* on certain biochemical parameter in Wister albino rats such as:

1-The effect of *Boscia senegalensis* leaves water extract on serum enzymes.

2- The effect of *Boscia senegalensis* leaves water extract on blood contents.

3- The effect of *Boscia senegalensis* leaves water extract on serum amino acids composition.

4- The effect of *Boscia senegalensis* leaves water extract on serum electrolytes.

Chapter One

Literature Review

1. 1 Medicinal plant

People have known the use of medicinal plants since ancient times. These plants have been used for the treatment of various diseases worldwide, because of ready availability, their low cost of production and less toxic action compared to those of synthetic compounds **(Asima and Pkrashi, 1995)**.

Africa is a continent endowed with an enormous wealth of plant resources over 5.000 distinct species are known to occur in the forest region most of them have been used for several countries in traditional medicine **(Maurice , 1993).**

In Sudan, numerous plants or plant products are commonly used by rural and urban citizen for the treatment of a wide variety of condition, often without prior research to prove their claim **(Bakhiet, 1995; Omer *et al.,* 1992 and Ahmed, 1988).**

1.2 *Boscia senegalensis* (Pers) Lam. ex Poiret

1.2.1 Taxonomy of *Boscia senegalensis:*

Kingdom	**Planate – Plants**
Subkingdom	**Tracheobionta – Vascular plants**
Superdivision	**Spermatophyta – Seed plants**
Division	**Magnoliophyta – Flowering plants**
Class	**Magnoliopsida – Dicotyledons**
Subclass	**Dilleniidae**
Order	**Capparales**
Family	**Capparaceae – Caper family**
Genus	**Boscia Lam. – boscia**
Species	**Boscia senegalensis (Pers) Lam. ex Poiret**

1.2.2 Common names

Aizen (sometimes spelled ayzen) is a Berber word and is the one most commonly used in the literature dealing with West Africa. Other names include mandiarha (Berber); mokheit, mukheit, umkheit (all Arabic), bere (Bambara); ngigili (Fulani).Other common names for the fruit include dilo (Hausa); bokkhelli and

kursan (hant, tadent, tadomet (Tamachek); harrenya (Sonrai); nabedega (More); and nkiandam and diendoum(Wolof)**(NRC,2008).**

1.2.3 Description of *B.senegalensis*:

Shrubs or small trees, up to 3 m high with stiff grey branches. leaves are alternative,cariaceous,petiolate ; laminas oblong-ellaptic to obovate, 3.8-10x1.7-4.9cm, apex apiculate or mucronate,base cuneate,margin entire ; petioles 0.5-1cm long. Inflorescences dense corymbs,, peduncles up to 3cm long ;pedicels 2-3 cm long,gynophores 2-4 mm long .Flowers greenish. Fruits drupe,globose, warted up to 1.3cm long ,pale yellow when ripe, 1-2seeded **(Elghazali *etal* .,1994).**

Figure1: Boscia senegalensis (flowering plant)

1.2.4 Habitat and Distribution

Boscia senegalensis **(**Pers.) Lam. ex Poiret: is distributed south of 16 N° **(Andrews, 1950).**

The aizen occurs across area that in recent decades has faced more hunger than any other in the world—the vast swath of Sahel and Sahara savannas stretching from Mauritania, Senegal, and Mali all the way to southeastern Egypt, Sudan, Ethiopia, Somalia, and Kenya **(NRS,2008).**

This plant's endurance is remarkable. It tolerates temperatures as high as 45°C, a level not rare in its habitat. It occupies most types of arid-land environment: stony slopes, sand dunes, and cracking-clay plains, just for starters. It often occurs in desiccated, barren, hard, and even fire-scorched sites. As to soils, they are usually poor, sandy, rocky, worn-out laterite, orclay. Commonly it sprouts directly out of termite mounds. And it survives in areas receiving as little as 100 mm annual rainfall, although it grows best where there is at least 250 mm. Contributing to the plant's built-in drought tolerance is its remarkable leaf structure the cuticle is up to 20 microns thick, the stomata are sunk in deep cavities, and each stomate has thickened walls and a protective armoring of papillae **(Killian , 1937).**

Figure2: Boscia Senegalensis (leaves & fruit)

1.2.5 Constituents of *B. Senegalensis*

The genus has been shown to elaborate several flavonoids

(Walter and Sequin, 1990).

Sesquiterpenes and their glycosides **(Pauli *etal*,1990)**, sulfur compound **(Kjaer *etal.*, 1973),** and lipid **(Grindley, 1948).**

Leaves constitute choline and sterols (β-sisterol, campesterol and stigmastrol) as well as aliphatic alcohole (predominantly C30,C34,C38),carbohydrates and four glycosinolates of unknown structure could be detected **(Kerharo and Adam,1974).**

Three of glucosinolate from leave as methyl and 2propylisothiocyanate and in trace amount 2-butylisothiocynate **(Kjaer *etal.*, 1973).** Proto alkaloid Lstachydrine and 3hydroxy from leave sample from Senegal **(Delaveau *etal.*, 1973).**

Root sample from Sudan give a positive reaction for sterol, triterpenes a negative reaction alkaloid cardilenpolides, tannin, saponin ,flavonoids and quinine

(ELkheir and Salih , 1980).

Saponine was detected in leave sample from Niger, alkaloid ,tannins, quinines and flavonoids were not present **(Baoua *et al*,1976).**

Methyl isothiocyanate which is liberated from a glucosinolate precursor; glucocapparin found in the fruits and leaves **(Seck, et al. 1993).**

1.2.6 Nutritional value

Detailed nutritional analyses seem unavailable, but the fruits are reportedly rich in calcium, phosphorus, iron, and B vitamins. They are also said to contain a little protein **(NRC, 2008).**

Seeds of ***B. senegalensis*** (dilo in Niger) Contained 70mg/100g from essential fatty acid, linolic acid.Ca 29mg/100 g dry Wt Mg 4.2/100g and traces copper selenium manganese. Zinc Iron however were presented at relatively high level ,(6.0 and 5.3mg/100 dry wit) respectively .the chemical composition of dilo harvested in Niger differs significantly from that of ***B. senegalensis*** seeds grown in Sudan.

(Kim and Pastuszyn ,1997).

The nutritional value of seeds of mukheit **(*Boscia sene***galensis (Pers) Lam ex Poir) and maikah (Dobera roxburghi Planch) widely used as food in times of famine in western Sudan have been investigated. The chemical composition of each food before and after the traditional debittering process is reported. Both foods compared favourably with the local staple cereals as regards the content of soluble carbohydrate and crude protein with values of 690 and 250 g kg-1 respectively for mukheit debittered by soaking in water at ambient temperature and 8 ,150 g kg-1 respectively for maikah debittered by boiling in water. The nutritional quality of protein in mukheit debittered by soaking in water at ambient temperature appeared similar to that of sorghum but was markedly enhanced if debittering was performed by boiling, although the crude protein content of the food fell substantially during the latter procedure **(Salih and Nour ,1991).**

Un- debittered : Protein (crude) = 29.3%. Oil = 0.7%. Ash = 3.5%. Fibre (crude) = 2.7%. Carbohydrate (soluble) (starch) = 39.5%; (sugars): Sucrose = 4.3%. D-glucose = 0.2%. D-fructose = 0.7%. Amino acids (g [16g N]-1): Aspartic acid = 7.7g. Threonine = 1.7g. Serine = 2.3g. Glutamic acid = 9.0g. Proline = 6.5g. Glycine = 3.5g. Alanine = 3.2g. Valine = 4.5g. Cysteine (performic acid oxidation) = 1.3g. Methionine (performic acid oxidation) = 1.8g. Isolelucine = 2.9g. Leucine = 7.0g. Tyrosine = 2.3g. Histidine = 1.3g. Lysine = 1.5g. Arginine = 15.1g. Minerals: Sulphur = 2.20mg/kg-1 (dry). Potassium = 0.15%-1 (dry). Magnesium = 0.10% (dry). Calcium = 0.14% (dry). Na = 0.01% (dry). K = 1.03mg/kg-% (dry). Zinc = 42mg/kg-1 (dry). Iron = 10.5mg/kg-1 (dry). Manganese = 17mg/kg-1 (dry). Copper = 8mg/kg-1 (dry) **(Abdelmuti ,1991).**

1.2.7 Uses of *B. senegalensis*

1.2.7.1 Nutritional uses

The mixing a suspension of powder prepared from the leaves or roots with cereal flour (or porridge) results in sweet products **(Salih and Nour , 1991).**

The rationale behind the use of these leaves for the production of foods with improved taste (through probably release of reducing sugars) has never been elucidated **(Dicko *etal* ., 2001).**

According to a Sudanese famine-food specialist, Aizen was the “number one” famine food during the horrific 1984 famine in the western Sudan. “It proved to be people’s lifesaver,” he reports, “and it saved more lives than all the food aid that was given. **(Abdelmuti ,1991)**

In famine times, people in rural Sudan rely on aizen. Typically, they collect the fruits; sun-dry them, separate the pea-sized seeds, and remove the hard outer seed coat. Seeds are then subjected to "sweetening" to remove bitter and possibly toxic components.

The traditional procedure involves soaking for a week, changing the water every day. Sometimes "kambo," local potash prepared from plant ash, is added to aid the debitteing. Less commonly, sweetening is conducted by boiling for 3 hours, with the water changed hourly. After such treatment, the sun-dried "sweet" seeds are stored until required, at which time they are boiled until soft, changing the water once during the process. The resulting food is usually eaten with oil and salt. Alternatively, seeds are ground to flour which is consumed in the form of kisra, flat thin bread popular in Sudan or Asida, a local form of porridge. The taste of the final product can be improved by blending with millet or sorghum flour **(NRC, 2008).**

1.2.7.2 Medicinal uses

The leaves of ***B. senegalensis*** are traditionally used for human and animal nutrition, protection of cereals against pathogens, and pharmacologic purposes

(Salih and Nour , 1991).

1.2.7.2.1 Traditional medicine

In senegal *Boscia senegalensis* used to treat colic without acting as a purgative, and for sever Jaundice and feverish cholecyctitis. Also it used as antihelmentic (Schistosoma) by decoction of leave ,for itching eye. Wolof treats cold with leave of ***B senegalensis*** also for catarrh in horse; pulverise the leave and rub into the nose or let animal inhale the vapor of the heated leave. A greul of leave or flour a medicine

or heamorrhoid is made by adding Salvadora persicaleave to that of ***B. senegalensis*** (eat every day). In Cameroon: plant is used for chronic ulcer, syphilis , colic and filariases .as a fumigation the leave are said to relive cough in horses. In Niger : hausa women take the powder of leave during the first 7 days of having birth, also use leaves powder or roots in the form of watery past to ulcer and eczema. the root are considered to be a medication for male sexual weakness .for exhaustion and fatique the decoction of the above ground leave plant part is drunk .leave powder in food is a cold medicine for horses and monkey.In Chad Bagirmi: wound and skin rashes are treated with bark extract, tooth ache and gingivitis with root infusion (mouth wash). In Sudan Kordofan province leave infusion is widely used medication for ventral disease, inflamed eye are also bathed with it .Root prepared are used for Jaundice. Root ,leave and bark are used as coagulating agent to clarify water .in west of Sudan and blue Nile ***B senegalensis*** is considered as one of the potent plant for treated ,turbid water .Water coagulation is believed to protect from disease like;

diarrhea ,gastro-intestile disorder ,gastric fever **(Neuwinger,1996).**

1.2.7.2.2 Recent medicine

1.2.7.2.2.1 Role against pathogen

B. senegalensis possesses antimicrobial and anti fungal activities

(**Almagboul *etal* .,1988 ; Laurens, 1985).**

It has been show to be effective uterine stimulation **(Bullough *etal.,* 1982).** The leave used in the preparation of malaria remedy and for the treatment of jaundice, fungal infection and viral diseases ,it applied externally for wound .The fruits

and roots are used as aphrodisiac, and the roots decoction is used for stomach ache and to facilitate labour **(Maurice,1993).**

Activities of a-amylase, B-amylase, exo-(1-3, 1-4)-B-D glucanase, and endo-(1-3)-B-D-glucanase were detected in leaves of ***Boscia senegalensis*** **(Dicko *etal,* 2001)**.

Synthesis of (1→3)-B-D-glucanases is enhanced in response to pathogen infection or a biotic stress condition **(Ryals *etal* ., 1996).**

(1→3)-B-D-glucanse are associated with antitumor, antibacterial, anti coagulator, and wound healing properties the soluble ones are the most active

(Bohn and BeMiller, 1995).

1.2.7.2.2.2 The effect on blood cells

Leaves contain the alkaloids L-stachydrine and hydroxy-3 stachydrine. Stachydrine affects aggregation of Thrombocytes and shortens the bleeding time **(DFID *etal*., 2009).**

1.2.7.2.2 .3 Anti inflammatory effect

Boscia senegalensis is a bush of African savannah, used in folk Medicine for the treatment of articulary pain. We have tested the anti-inflammatory action of an aqueous and alcoholic extract of leaves in rats , the aqueous and alcoholic leaves extract (400 mg/kg) . The aqueous and alcoholic extracts at dose of 400 mg/kg inhibited

respectively the development of oedema. The extract showed the presence of

alkaloids , saponosides, flavonoids and tannins on phytochemical screening. These findings indicate that the leaves extracts of ***Boscia senegalensis*** contain anti-inflammatory active principles for the acute inflammation treatment. Moreover, these results justify the use of the leaves of ***Boscia senegalensis*** for the treatment of many inflammatory diseases **(Nongoniermsierm , 2007).**

1.2.7.3 Pest control

The biocide activity of the leaves on insects has been justified by the presence of volatile compounds such as methyl lisothiocyanate and mehylcyanide **(longoay *etal*., 1994).**

Fresh ground leaves caused 100 percent mortality in adult *C. maculatus* within 24 hours and prevented egg-laying. Admixture of fresh whole leaves and dry leaf powder was less effective, Acetone extract of fruits a glass desiccator caused 100 % mortality in adult S. cereallela within 1.5 hours, the LT50 values for C. maculatus and P. truncatus were 2.3 hours and 3.8 hours respectively **(Seck *et al*., 1993).**

1.2.7. 4 Role in storage

The effect of hermetic storage alone or in combination with ***B. senegalensis*** has been evaluated against *Callosobruchus maculatu*s. Analysis of gas concentrations within a 7-day period indicated that O2 declined from 19.2 to 2.3% and CO2 rose from 1.2 to 22.8%. Prolonged storage durations increased adult mortality, signifi-cantly increased the developmental time and induced 60–80% reduction in the F1 progeny. The use of hermetic storage in combination with ***B senegalensis*** fruits, at

1.2 g/l (flask volume) reduced the emergence of the cowpea beetle, while 2.4–4.8 g/l completely inhibited the production of a new generation of C. maculates

(Seck *et al*., 1995).

1.2.7.4 Water clarification

Aizen contains natural coagulants. In Sudan, Niger, and Nigeria, for example, bark, twigs, leaves, and roots are used to scavenge suspended and colloidal compounds from unclean water (such as that from ponds churned up by storms or from baobab-tree cisterns contaminated with soil). Normally the plant parts are sliced up and placed on the water surface. Compounds leach out and catch the clay and other particulates like magnets, causing them to clump and settle to the bottom. It is reported that truly turbid water can be safely drunk after just a day of such treatment. For even faster results, aizen branches are swirled in the water. Indeed, certain Tuareg groups in the Sahara region fill sacks with aizen leaves and dunk them into the muddy pools that comprise their only source for drinking. Following a rare desert downpour they may also place these giant tea bags across ditches so that the runoff clarifies itself as it oozes through

(NRC, 2008).

Boscia senegalensis is reduced turbidity of water by 94.26%,many factors were effect the efficiency of plant ,such as the methods of coagulant ,the dose ,filtration and extraction time (long contact time led to deterioration on water quality **)**

(ELgaddal , 1993).

1.2.8 Toxicity

The nature glycosinolate proved to be a source of anti thyroid drug; the inhibit the organic binding of iodine in the thyroid gland, enlarge the thyroid and cause goiter.β sisterol is it show a significant anti inflammatory and antipyretic activity similar to acetyl salysilic acid by oral administration. Now it fails to induce gastric ulceration or it is analgesic activity **(Gupta *et al.*, 1980).**

β sisterol posses anti bacterial effect **(Chi and choi , 1985).** In laboratory animal β sisterol in female may cause abortion **(Burck *etal* .,1982; Pakarashi and Basak 1976)**,it can exert oestorogenic effect, In male β sisterol produced an antifertility effect (it decreases the testicular weight and sperm concentration significantly with high doses ,but also after long term treatment with a low dose **(Malani and Vanitha Kumari,1991).**

Phytosterol fraction it is used for hyper cholestremia for avoid gallstones, it combination with chenodeoxy-cholic acid for cholelitholysis;it cause increased excretion of cholesterol **(Neuwinger,1996).**

Leafless twigs contain glucosinalates, which can hydrolyze to mustard oils, which are highly toxic and irritant to mucous membranes **(DFID *etal*., 2009)**.

Chapter Two

Materials and Methods

This work has been conducted in the Department of Pharmacology and toxicology in Medicinal and Aromatic Plants Research Institute (MAPRI), National Center for Research, Khartoum and Research Laboratory Unit, Khartoum Teaching Hospital, and Central laboratory.

2.1 Materials:

2.1.1 Experimental animals:

Healthy Wister albino rats males obtained from the National Center for research were used in this study. The rats were housed identically in stainless steel cages in a room under suitable conditions. Twenty four rats were divided into three groups on the basis of body weight of each group and average weight of rat in each group was 75g.

2.1.2 Plant material:

Boscia senegalensis leaves were collected from western of Sudan (Om Rawabah) .The leaves of ***B sengalensis*** authenticated by botanists in herbarium of Medicinal and Aromatic Plants Research Institute, Khartoum.

2.1.2.1 Preparation of the aqueous extract:

500 ml of hot distilled water was added to the 100 g of each plant sample and left till cooled down with continuous stirring at room temperatures. Extract was then filtered and stored in a deep freezer till freezed. Freezed extract was freezed-dried using freezdrier (Trivac, USA) till all of the ice was removed out and powdered extract was obtained. Yield percentage was calculated **(Harborne, 1984**).

1500 ml of hot distilled water was added to the 250 g of leaves of ***B. senegalensis*** sample and 29g were prepared. .

2.1.3 Experimental design:

The groups were designed as A, Band C and eight rats in each group.

-The rats of group A were kept as normal control.

-The rats of groups B, C were served as treated animals.

2.1.4 Feeding programme:

Each group was kept in a cage and was supplied with water and basal diet of which each kilogram was composed of 725g meat and 240g flour plus 1g salt. The feed was available at the rate of 35g per day in each cage. The three groups were fed as follow:

Group A: served as control, received basal diet (BD) only.

Group B: received (BD) and orally 200 mg/kg body weight (BWt) ***B Senegalensis's*** leaves water extract.

Group C: received BD and orally 400 mg/kg (BWt) ***Boscia senegalensis*** leave water extract.

2.1.5 Instruments:

-Finn pipette , and digital micro pipette , was used for pipetting of serum sample.

-Capillary tubes

-Anti coagulant blood containers were used for blood collection for complete hemogram.

-Sterile plane containers were used for keeping serum.

2.1.6 Sample collection:

Blood sample were collected from all group (4 from each one by using capillary tube) to determine complete heamogram. After 15 days rats was slaughtered and the blood was been collected on plain containers:

1- The blood was centrifuged at 5000 rpm for 10 minutes. Then serum were separated and used to estimate some biochemical parameters.

2- Blood for heamatology.

3-Some of rats were examined to identify gross lesions and specimens of the liver, Kidneys, heart, spleen and intestines which were fixed in 10% neutral buffered formalin and processed for histopathology.

2.2 Methods:

2.2.1 Laboratory analysis:

2.2.2. Haematological methods

Blood samples were collected from optimal vein or by slaughtering animals and immediately placed into dry -cleaned tubes containing Ethylene Diamine Tetra acetic Acid (EDTA) as an anticoagulant. The hematological analysis was done by the mean of Sysmex -KX-21 N. Haematological parameters: Haemoglobin (Hb), Red blood cells (RBCs),White blood cells (WBCs), Mean corpuscular haemoglobin concentration (MCV) ,and (MCHC) were determined.

Sysmex-Kx-21, Japan, 1999:

This Analyzer is an automatic multi-parameter blood cell counter for in vitro diagnosis use in clinical laboratories. The Sysmex KX-21 processes approximately 60 samples/hour and are displayed on the LCD screen, the particle distribution curves of WBC, RBCs, differential WBCs counts and platelets count along with data of other parameters.

CD Analyzer detection method:

Blood sample is aspirated, measured to determine volume, diluted at specified ratio, with transducer use. The transducer chamber has minute hole called the aperture, on both sides of it, there are electrodes between which direct current flows. Blood cell suspended and diluted sample passes through the aperture causing direct current resistant to change between the electrodes as direct resistance changes, the

blood size is detected as electric pulses. The parameters measured were Hb, RBCs and erythrocyte series, MCV, MCH and MCHC.

2.2.3 Sero biochemical methods:

Blood samples were collected and allowed to clot and sera were separated by centrifugation at 5000 r.p.m for 10 min and stored at -20°C until analyzed.

A-Some of sera parameters were estimated using the Roche diagnostic Hitachi 902 Analyzer. In Research and Laboratory Unit, Khartoum Teaching hospital .

B- Other sera were estimated using The SYKAM system The Amino Acid Analyzer S433, Done by The Central laboratory.

Roche diagnostic/Hitachi 902 analyzer:

It is an analyzer to report test results on various body fluid samples for wide range of analysis. It is fully automated, computerized, performs potentiometric and photometric assays, and includes analytical processing unit and luminance crystal display (LCD) touch screen, with a standard printer to print the results. The analyzer is characterized by doing 200 photometric tests/ hour, and refrigerated storage for 40 reagent containers, as well as it has end point, and kinetic and isoenzymes reactions.

2.2.4 Determination of alkaline phosphatase ALP:

Principles:

Under alkaline condition, p-nitrophenol is converted to 4-nitrophenoxide, which develop a very intense yellow color. The intensity is proportional to the activity of

alkaline phosphtase.

p-nitrophenyl phosphate + H_2O $\xrightarrow{\text{alkaline phosphatase}}$ phosphate + p-nitrophenol

Reagents:

Reagents1:

2-Amino-2-Methyl-1-Propanol	0.9 mol/l
pH	10.4
Magnesium acetate	1.6mmol/l
Zinc sulphate	0.4 mmol/l
EDTA	2 mmol/1

Reagents2:

P-nitrophenyl phosphate	16 mmol/l

Procedure:

The analyzer automatically added an equal quantity from sample, R1 and R2 to the reaction.

Calculations:

The analyzer automatically calculated the analytic concentration of each sample. The result appeared directly on the screen of the diagnostic machine at the end of the reaction between the reagents and samples.

2.2.5 Determination of Alanine amino transferase (ALT):

Principles:

ALT catalyses the transformation of L-alanine and 2-oxoglutarate at optimal pH . The pyruvate released in the reaction is transformed by Lactate dehydrogenase (LDH) in the presence of NADH/NAD ˙coenzyme to L-lactate .While the NADH/NAD oxidoreductive process shows a decrease in absorbance at 340nm .The change in absorbance correlates with serum ALT activity.

L-Alanine + α-Ketoglutarate $\xrightarrow{\text{ALT}}$ pyruvate-L-Glutamate

Pyruvate + NADH $\xrightarrow{\text{LDH}}$ L-Lactate-NAD

Reagents:

Reagents (R1)

Tris buffer, pH: 7.50	110 mmol/L
L-Alanine	600 mmol/L
LDH	1500u/L
NADH	240 mmol/L

Reagents (R2)

α-Ketoglutarate	16 mmol/L

Procedure:

The analyzer automatically added an equal quantity from sample, R1 and R2 to the reaction.

Calculations:

The analyzer automatically calculated the analytic concentration of each sample. The result appeared directly on the screen of the diagnostic machine at the end of the reaction between the reagents and samples.

2.2.6 Determination of Aspartate amino transferase (AST):

Two substrate participate in the reaction catalyzed by AST .L-aspartate and oxolglutarate (With the help of NADH coenzyme) .Malate dehydrogenase (MDH) contained in the reagent catalyses the transformation of oxalacetate released in the first reaction .The oxido-reductive process of NADH/NAD is indicated by a decrease in absorbance at 340 nm.The lactate dehydrogenase (LDH) in the medium counte-racts the disturbing effect of pyruvate contained in the sample.

L-Aspartate + α-Ketoglutarate $\xrightarrow{\text{AST}}$ L-glutamate -Oxalacetate

Oxalacetate + NADH $\xrightarrow{\text{MDH}}$ L-Malate -NAD

Reagents:

Reagents (R1)

Tris buffer, pH: 7.80	88	mmol/L

L-Aspartate	260 mmol/L
LDH	1500 u/L
MDH	900 u/L
NADH	0.24 mmol/L

Reagents (R2)

α-Ketoglutarate	12 mmol/L

Procedure:

The analyzer automatically added an equal quantity from sample, R1 and R2 to the reaction.

Calculations:

The analyzer automatically calculated the analytic concentration of each sample. The result appeared directly on the screen of the diagnostic machine at the end of the reaction between the reagents and samples.

2.2.7 Determination of Creatinine:

Principle:

Creatinine under alkaline conditions reacts with picrate ions forming a reddish complex. The formation rate of the complex measured through the increase of absorbance in a prefixed interval of time is proportional to the concentration of Creatinine in sample.

Reagents:

Picric acid	40 mmol/l
Potassium ferricyanide	40µmol/l
Phosphate buffer	300mmol/l
Creatinine Standard	177mmol/l

Procedure:

The analyzer automatically added an equal quantity from sample, R1 and R2 to the reaction.

Calculations:

The analyzer automatically calculated the analytic concentration of each sample. The result appeared directly on the screen of the diagnostic machine at the end of the reaction between the reagents and samples.

2.2.8 Determination of urea:

Urea concentration in serum measured using enzymatic colorimetric method described by **Monica (1992).**

Principle:

Urea +2 H_2O —Urease→ 2 NH_4 + 2HCO_3

NH_4 +2-Oxoglutrate + NADH —*GLDH*→ L-Glutamate + NAD +H_2O

Reagents:

Reagents1:

Tris buffer	120 mmol/l
pH	7.8
2- Oxoglutrate	7 mmol/l
ADP	0.6 mmol/l
Urea	1 mmol/l
Urease	≥ 5 KU/l
Glutamate dehydrogenase	≥ 1 KU/l

Reagents2:

NADH	0.25 mmol/l

Procedure:

The analyzer automatically added an equal quantity from sample, R1 and R2 to the reaction.

Calculations:

The analyzer automatically calculated the analytic concentration of each sample. The result appeared directly on the screen of the diagnostic machine at the end of the reaction between the reagents and samples.

2.2.9.1 Measurement of Serum Sodium & Potassium

Sodium:

Serum sodium was determined by flame-photometry (Evans Electro selenium Ltd., England) as described by **(Varley ,1967)**. The method is based on passing under controlled conditions, diluted serum (1: 100) as a very fine spray in the air supply to a burner where the solution evaporates and the salt dissociates to give neutral atoms. Light of characteristic wavelengths was emitted and passed through aspecific filler for sodium on to a selenium cell and the amount of current produced was read on a galvanometer. Readings were recorded and values were estimated in mEq/ liter as follows:

$$\text{Concentration (Meq/l)} = \frac{\text{T- sample X 140}}{\text{S- sample}}$$

The values were then converted to mg/dl.

Potassium:

The method described by **(Varley ,1967)** for the determination of serum potassium is based on the same principle of flame-photometry for serum sodium. The sensitivity of the instrument was varied in order to use the same dilution of serum (1:100) for both sodium and potassium.

Light of characteristic wavelengths was emitted and passed through specific filter for potassium on to a selenium cell and the amount of current produced was measured.

Changes in galvanometer readings were recorded and values were estimated in meq/l as follows:

$$\text{Concentration (Meq/l)} = \frac{\text{T- sample} \quad \text{X } 5}{\text{S- Standard}}$$

The values were there converted to mg/dl.

2.2.10 Determination of serum amino acids: Analyzed by Amino Acid Analyzer S433 (Central laboratory):

2.2.10.1 The Amino Acid Analyzer S433:

It is an analyzer to report test results on physiological fluid samples and hydrolyzed sample to determine amino acid. It is fully automated, computerized, and includes analytical processing unit and luminance crystal display (LCD) touch screen, with a standard printer to print the results.

It put together from 4 modules: The sample Injector 5200 controls the complete system and gives the signal to the Amino Acid Reaction Module S4300 and the Solvent Delivery System S2100 for starting installed programs.

Preparation of sample:

1-prepared 20% solution of 5- Sulfosalysilic acid

2-Added 100 of 20% solution of 5-sulfosalysilic acid to 900ml of serum sample.

3-Incubated at 4C° for half an hour.

4-Centerfugeated 13000pmfor10-15min

5-Took superament and diluted by sample diluting buffer .The precision and accuracy of all methods used in this study were checked each time a batch was analyzed by including commercially prepared control sera.

Reagent

The ninhydrin reagent the most commonly used for amino acid determination.

Usually consist the following:

1-Crystalline ninhydrin

2-An organic solvent to dissolve the hydrin

3- Strong buffer solution to reach the optimum PH value for the ninhydrin reaction.

2.2.11 Histopathological methods:

Tissue specimens collected from animals liver, kidney, heart, lung, spleen and intestine after dead or slaughtered were immediately fixed in 10% buffered formalin (Sodium hydrogen 6.5 gm/L and sodium dihydrogen 4 gm/L) then embedded in paraffin wax, sectioned at 5µ and stained routinely with Haematoxylin and Eosin (H&E) using (**Drury and Wallington ,1980)** method.

2.2.12 Quality control:

The precision and accuracy of all methods used in this study were checked each time; a batch was analyzed by including commercially prepared control sera.

2.2.13 Statistical analysis

According to complete randomized design the rats were divided into three groups (A, B and C) each group contain 8 rats for similar body weight.

Mean values obtained in blood and serum parameters were statistically verified using t-test. All means were compared at probability level less than 5% **(Mendehall, 1971)**.

Chapter three

The Results

3.1 The effects of oral administration of *Boscia senegalensis* water extract on blood contents (Hb, RBCs ,WBCs ,Platelets, MCV,MCH and MCHC).

3.1.1 After one week of treatment:

B .senegalensis leaves water extract on two different concentrations (B and C) when administrated orally to normal rats had no significant effect either on Hb, Platelets , RBCs , WBCs , MCV,MCH or MCHC .But**,** all of them had been mild decreased (in contrast WBC which was increased) when compared with control. As shown in table 1.

Table 1-The effects of oral administration of *Boscia senegalensis* water extract on blood contents (Hb, , RBC , WBCs ,Platelets ,MCV,MCH and MCHC).
After one week of treatment:

Parameter	Group A	Group B	Group C
WBC µl	7.2±0.8297	7.8±0.5	9.590±1.79
Hb g/dl	155±5.9	155±3.5	152.50±2.179
RBCs µl	9.31±0.48	9.31±0.11	9.935±0.458
Plt µl	1170±107.21	1042±19.37	1077±59.545
MCV fL	64.5±1.04	64.7±063	64.5±1.04
MCH Pg	16.70±0.40	16.63±.2658	15.425±.6102
MCHC g/dL	259.25±2.72	256.50±3.97	239±6.9

Data are expressed as Means ±standard error
* statistical difference $p<0.05$
Group A: Normal control rats
Group B: Normal rats administrated with(200mg/kg) from *Boscia senegalensis* leaves water extract .
Group C: Normal rats administrated with (400mg/kg) from *Boscia senegalensis* leaves water extract .

3.1.2 After three weeks at the end of experiment:

B. senegalensis water extract on the different two concentrations (B and C) when administrated orally to normal rats had significant effect on Hb and RBCs , there were an increase on Hb and Decrease on RBCs when compared with control .As shown in table 2.

Table 2- **The effects of oral administration of *Boscia senegalensis* water extract on blood contents (Hb, RBCs,WBCs ,Platlets, MCV,MCH and MCHC)**. After three week of treatment :

Parameter	Group A	Group B	Group C
WBCs µl	14.8±3.398	14.4750±2.61	10.1±1.84872
Hb g/dL	7.26±0.204	15.35±0.395*	12.2±1.330*
RBCs µl	13.7±0.218	7.598±02153*	6.09±06414*
Plt µl	811±177.8	797.50±40.590	561±95.496
MCV fL	65±2.131	65±0.707	65.33±0.667
MCH pg	20.05±0.4975	20.425±0.45758	19.30±0.7506
MCHC g/dL	30.85±0.7837	31.25±0.4406	26.96±3.5825

Data are expressed as Means ±standard error

* statistical difference $p<0.05$

Group A: Normal control rats

Group B: Normal rats administrated with (200mg/kg) from *B. senegalensis* leaves water extract .

Group C: Normal rats administrated with (400mg/kg) from *B. senegalensis* leaves water extract

3.2 The effects of oral administration of *B. senegalensis* leaves water extract on serum AST, ALT , ALP Enzymes ,Urea and Creatinine :

3.2.1 After two weeks of experiment:

B. senegalensis leaves water extract in the different two concentrations when administrated orally to normal rats had no significant effect either on serum AST, ALT, ALP concentration or on serum Urea and Creatinine on treated groups (B and C) , but ALT and AST concentration on (B and C) had been numerically increased when compared with control (group A).However Urea and Creatinine had been decreased**.** As shown in table 3.

Table 3- **The effects of oral administration of *B. senegalensis* water extract on serum AST, ALT ,ALP Enzymes ,Urea and creatinine ,after 2weeks of treatment:**

Parameter	Group A	Group B	Group C
ALT U/L	37±2.3	38±2.0	41±2.6
AST U/L	127±4.6	155±11.6	158±5.3
AlP U/L	256±38	150±23	212±21
urea mg/dl	36.7±2.6	34.5±2.8	4.6±2.3
Creatinine mg/dl	0.57±0.02	0.55±0.02	0.52±0.02

Data are expressed as mean ±standard error
Mean value significant having star (*) supscripts within the same row ,statistical difference $p<0.05$
Group A: Normal control rats
Group B: Normal rats administrated with (200mg/kg) from *Boscia senegalensis* leaves water extract .
Group C: Normal rats administrated with (400mg/kg)from *Boscia senegalensis* leaves water extract

3.2.2 After three weeks of experiment:

B. senegalensis water extract in the different two concentrations when administrated orally to normal rats had no significant effect either on serum AST, ALT , ALP concentration or on serum urea and Creatinine on treated groups(B and C) ,but ALP, ALT ,AST and urea concentration OF (group B) had been numerically decreased when compared with control (group A) . How ever, creatinine had been increased. By contrast on group C, ALP, ALT and AST had been increased. As shown in table.

Table 4- **The effects of oral administration of *B. senegalensis* water extract on serum AST, ALT ,ALP Enzymes ,Urea and creatinine :**After three weeks:

paramater	Group A	Group B	Group C
ALT U/L	42.75±6.8	41.50±2.363	46.33±4.177
AST U/L	183.50±9.179	157.25±10.0	199±8.353
AlP U/L	174±24.028	142±35.267	225.33±36.375
urea mg/dl	43.25±5.154	42.5±5.1	43.0±2.517
creatinine mg/dl	0.70±0.04	0.75±0.06	0.70±0.0577

Data are expressed as Means ±standard error

* statistical difference $p<0.05$

Group A: Normal control rats

Group B: Normal rats administrated with (200mg/kg) from *Boscia senegalensis* leaves water extract .

Group C: Normal rats administrated with (400mg/kg) from *Boscia senegalensis* leaves water extract .

3.3 The effects of oral administration of *B. senegalensis* water extract on serum electrolytes:

3.3.1 The effects of oral administration of *B. senegalensis* water extract on serum electrolyte after two weeks:

B. senegalensis water's extract in the different two concentrations when(B and C) administrated orally to normal rats had no significant effect either on serum sodium or potassium ,but either on B or C group Sodium had been numerically increased ,Potassium increased when compared with control (group A) As shown in table5.

Table 5- **The effects of oral administration of** *B. senegalensis water* **extract on serum electrolytes: after 2weeks of treatment:**

Parameter	Group A	Group B	Group C
SODIUM mmol/l	125.75±2.250	127.25±2.213	129.75±1.109
POTASSIUM mmol/l	5.650±0.2723	5.850±0.1658	6.075±0.3614

Data are expressed as Means ±standard error

* statistical difference $p<0.05$

Group A: Normal control rats

Group B: Normal rats administrated with (200mg/kg) from *Boscia senegalensis* leaves water extract .

Group C: Normal rats administrated with (400mg/kg) from *Boscia senegalensis* leaves water extract .

3.3.2 The effects of oral administration of *B. senegalensis* water's extract on serum electrolyte after three weeks:

B. senegalensis water's extract in the different two concentrations (B and C) when administrated orally to normal rats had no significant effect either on serum Sodium or Potassium , but all of them Sodium had been numerically increased and Potassium decreased when compared with control (group A). As shown in table 6.

Table 6- The effects of oral administration of *B. senegalensis water* extract on serum electrolytes: after3weeks of treatment:

Parameter	Group A	Group B	Group C
SODIUM mmol/l	136.50±4.735	137.00±2.198	146.67±4.667
POTASSIUM mmol/l	6.65±0.171	6.48±0.125	6.33±0.267

Data are expressed as Means ±standard error

* statistical difference $p<0.05$

Group A: Normal control rats

Group B: Normal rats administrated with (200mg/kg) from *B. senegalensis* leaves water extract .Group C: Normal rats administrated with (400mg

3.4 The effects of oral administration of *Boscia senegalensis* leaves water extract on serum amino acids on normal rats:

3.4.1 The effects of oral administration of *Boscia senegalensis leaves* water extract on serum amino acid on normal rats after two weeks:

The effects of oral administration of ***Boscia senegalensis*** leaves water extract on serum amino acid on normal rats' :treated rats(B and C) , serum contained a-amino butyric acid and Glutamic acid when compared with group A (control),.Taurine level showed an increase in group B and mild decreased on C when compared with control A. Spartic acid numerically decreased on treated rats (B and C) when compared

	Reten . Time{min}	Amount {umol\l}	Amount [%]	Compound Name

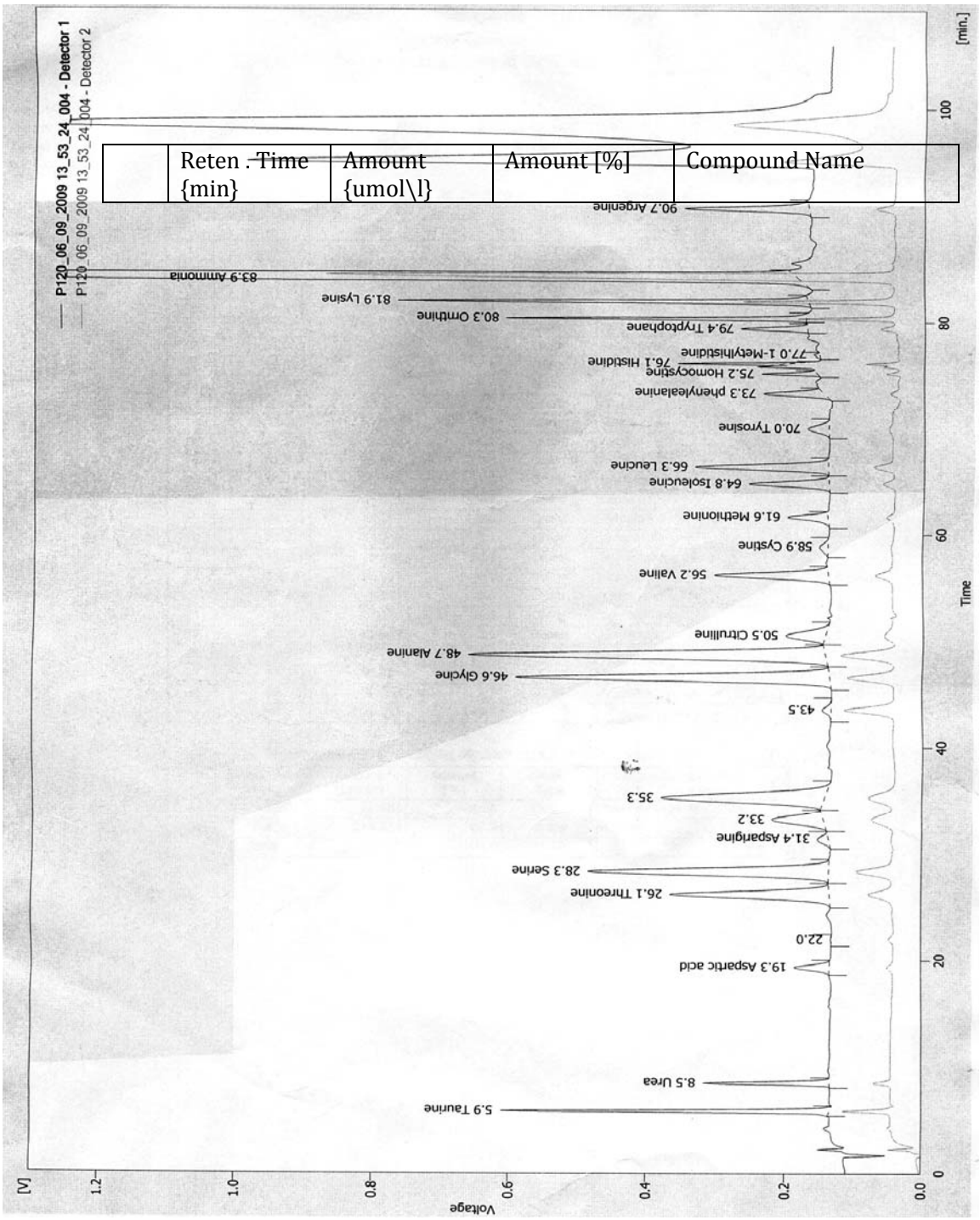
with control A. Therionine increased on treated rats (B and C) but elevation on B more than C. Serine numerically decreased on group C ,Sparagen showed no changes on C but an increase on group B. Glycine showed an increase on B . Alanine showed an increase on group C and decreased on B. Citruline decrease on B. Aminobutric acid showed an increase on group B more than C .Valine numerically decreased on C. Cystine no changes .Methionine numerically decreased on C. Isoleusine mild increased on B. leucine decreased on C and increased on B .Tyrosine increased on treated rats (B and C).Phenylalanine increased on B. Homocyctine mild decreased on treated rats B&C .Histidine mild increased on treated rats (B and C). Methyl histidine mild increased on B and decreased on C .Tryptophan mild decreased on C .Ornithin numericly decreased on B.lycine an increase on treated rats .Argenine increased on B. Hydroxyproline decreased on B , Proline decreased on two treated rats when compared with group A (Control) .As shown in tables 7,8 and 9. And figures 3, 4 and 5.

1	5.877	89.359	3.5	Taurin
2	8.531	697.711	27.1	Urea
3	19.331	11.477	0.4	Aspatric acid
4	26.149	104.388	4.1	Threonine
5	28.301	152.023	5.9	Serine
6	31.397	25.116	1.0	Asparigine
	46.595	200.020	7.8	Glycine
8	48.691	245.300	9.5	Alanine
9	50.520	32.782	1.3	Citruline
10	56.203	65.046	2.5	Valine
11	58.888	5.630	0.2	Cystine
12	61.648	20.342	0.8	Methionine
13	64.756	39.353	1.5	Isoleucine
14	66.339	68.304	2.7	Leucine
15	69.971	15,277	0.6	Tyrosine
16	73.328	27.249	1.1	Phenylealanine
17	75.157	8.147	0.3	Homocystine;
18	76.123	20.427	0.8	Histidine
19	77.008	1.943	0.1	1-Methylhistdine
20	79.405	27.273	1.1	Tryptophane
21	80.349	66.989	2.6	Ornthine
22	81.947	107.719	4.2	Lysine
23	83.941	480.335	8.6	Ammonia
24	90.653	63.563	2.5	Argenine
	total	2576.351	100.0	

Table7- Determination of serum amino acids composition on normal rats group A (Control) after 2 wee

	Reten . Time {min}	Amount {umol\l}	Amount [%]	Compound Name
1	22.021	22.681	16.1	HYdroxy-prolline
2	43.544	118.214	83.9	Proline
	Total	140.895	100.0	

Figure 3: Determination of serum amino acids composition on normal rats group A after two weeks of treatments



	Reten . Time {min}	Amount {umol\l}	Amount [%]	Compound Name

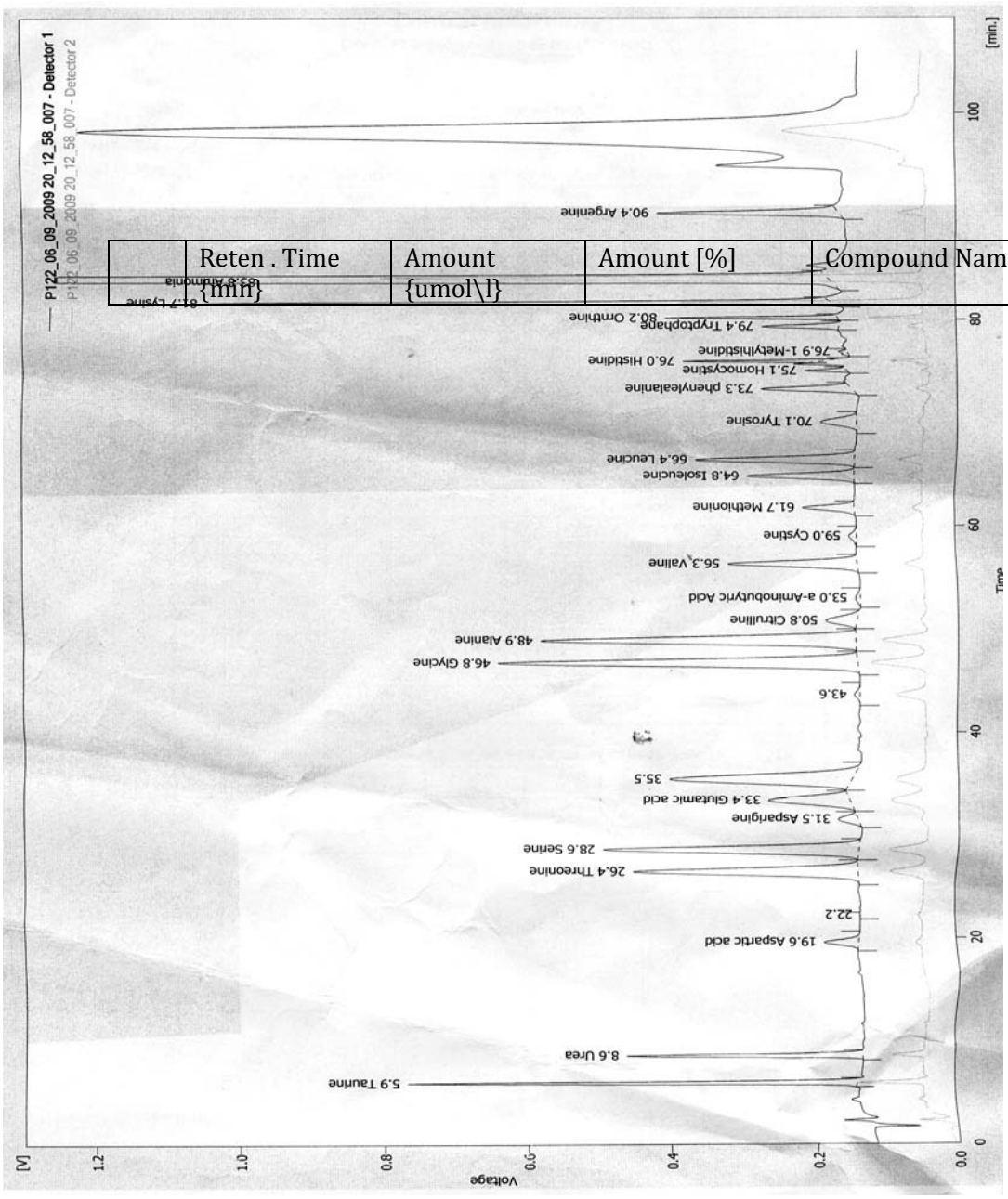
Table8- Determination of serum amino acids composition group B after 2 week

1	5.947	117.936	3.5	Taurin
2	8.621	1318.194	39.0	Urea
3	19.563	10.986	0.3	Aspatric acid
4	26.427	140.794	4.2	Threonine
5	28.565	149.289	4.4	Serine
6	31.512	40.519	1.2	Asparigine
	33.384	69.439	2.1	Gulatamic acid
8	46.763	229.949	6.8	Glycine
9	48.923	206.846	6.1	Alanine
10		23.176	0.7	Citruline
11	50.805	4.233	0.1	a-Aminobutyric Acid
12	52.973	68.886	2.0	Valine
13	58.997	4.615	0.1	Cystine
14	61.741	24.432	0.7	Methionine
15	64.813	50.398	1.5	Isoleucine
16	66.400	78.988	2.3	Leucine
17	70.080	24.342	0.7	Tyrosine
18	73.344	38.348	1.1	Phenylealanine
19	75.096	5.681	0.2	Homocystine;
20	76.035	26.331	0.8	Histidine
21	76.920	3.048	0.1	1-Methylhistdine
22	79.360	28.847	0.9	Tryptophane
23	80.216	33.713	1.0	Ornthine
24	81.733	157.704	4.7	Lysine
	83.757	442.283	13.1	Ammonia
	90.355	83.382	2.5	Argenine
	total	3382.358	100.0	

	Reten . Time {min}	Amount {umol\l}	Amount [%]	Compound Name
1	22.203	2.734	3.9	HYdroxy-prolline
2	43.632	68.069	96.1	Proline
	Total	70.803	100.0	

Group B: Normal rat administered with leaves water extract of B. senegalensis (200mg kg¯1)

Figure 4: Determination of serum amino acids composition on normal rats group B after two weeks of treatments:



	Reten . Time {min}	Amount {umol\l}	Amount [%]	Compound Name

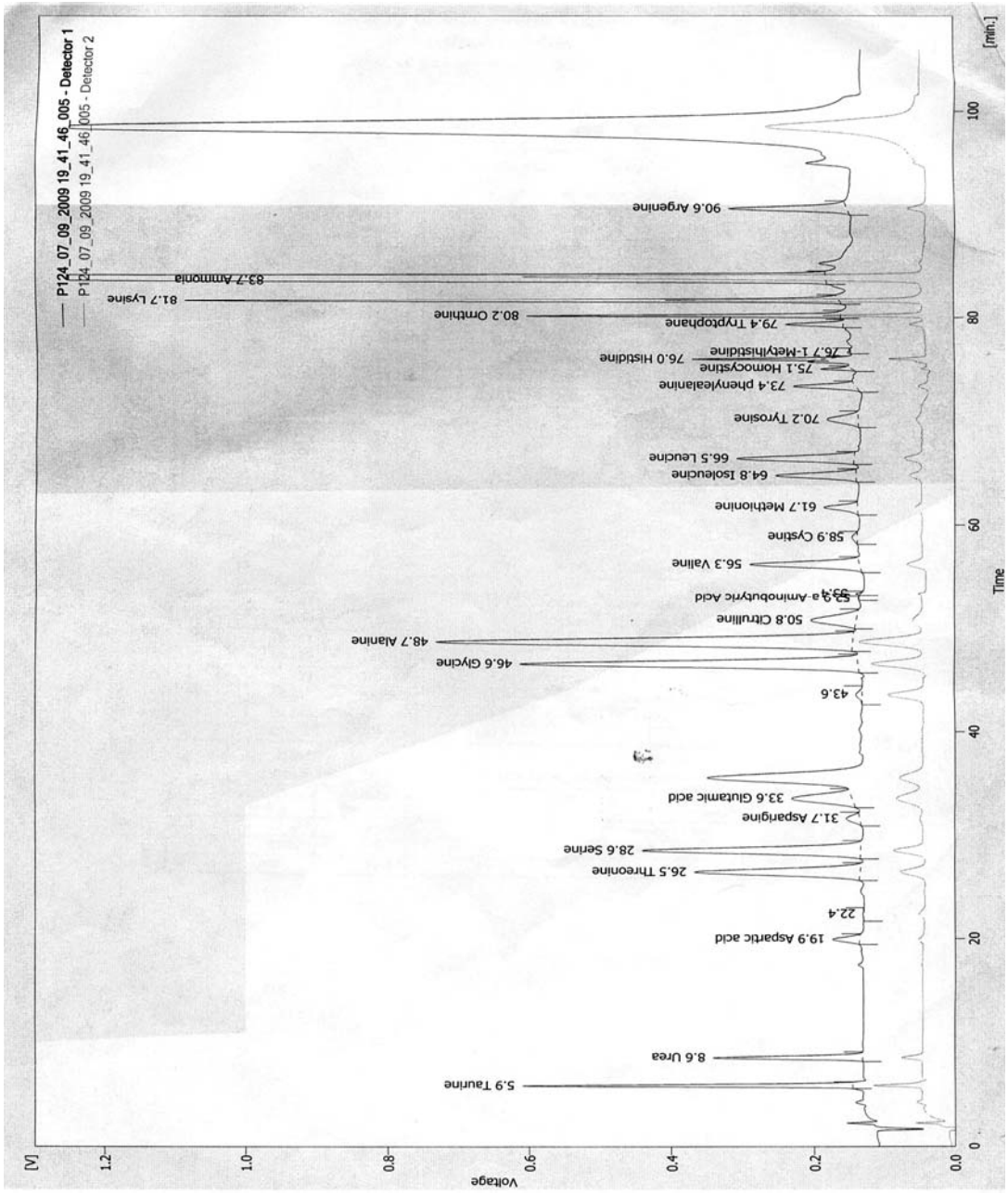
Table 9- Determination of serum amino acids composition group C after 2 weeks OF treatment:

1	5.893	83.484	3.0	Taurin
2	8.603	900.897	32.3	Urea
3	19.939	7.389	0.3	Aspatric acid
4	26.509	99.555	3,6	Threonine
5	28.592	120.671	4.3	Serine
6	3.661	26.990	1.0	ASparigine
7	33.608	50.284	1.8	Glutamic acid…
8	46.595	203.261	7.3	Glycine
9	48.717	285.855	10.2	Alanine
10	50.776	36.446	1.3	Citruline
11	52.933	0.179	0.0	a-Aminobutyric
12	56.256	56.851	2.0	Valine
13	58.872	2.600	0.1	Cystine
14	61.741	16.282	0.6	Methionine
15	64.800	38.319	1.4	Isoleucine
16	66.451	56.567	2.0	Leucine
17	70.237	20.976	0.8	Tyrosine
18	73.400	25.628	0.9	Phenylealanine
19	75.056	3.998	0.1	Homocystine
20	76.024	24.943	0.9	Histidine
21	76.659	0.727	0.0	1-Methlhistidine
22	79.381	22.375	0.8	Tryptophane..
23	80.227	65.079	2.3	Ornthine
24	81.739	161.057	5.8	Lysine
25	83.717	418.970	15.0	Ammonia
26	90.632	60.040	2.2	Argenine
	total	2789.421	100.0	

	Reten . Time {min}	Amount {umol\l}	Amount [%]	Compound Name
1	22.427	17.662	19.1	HYdroxy-prolline
2	43.579	74.914	80.9	Proline
	total	92.575	100.0	

Group C: ***Normal rat administered water extract of B. senegalensis (400mg kg^{-1})***

Figure 5: Determination of serum amino acids composition on normal rats group C after two weeks of treatments:



3.4.2 The effects of oral administration of *Boscia senegalensis* leaves water extract on serum amino acids on normal rats three weeks:

No sparagene was present on (group C) and no a-minobutyric acid on B group when compare with group A(control) Taurine level showed mild decrease in group B and numerically decreased on C when compared with control A. Spartic acid mild decreased on treated rats(B and C) when compared with control A. Therionine increased on B and decreased on C . .Serine numerically decreased on group C. Glutamic acid increased on group C .Glycine showed an increase on B .Alanine showed an increase on group C and decreased on B. Citruline numerically decreased on B . Valine mild decreased on B and mild increased on C. Cystine numerically decreased on B and increased on C.

Methionine no change. Isoleusine mild increased on B and mild decreased on C. leucine numerically increased on B and decreased on C .Tyrosine numerically increased on treated rats (B and C).Phenylalanine increased on B.Homocyctine no changes on treated rats (B and C) .Histidine numerically increased on treated rats (B&C),Methyl histidine no found on C only on B.

Tryptophan mild increased on C no change on B .Ornithin numerically increased on treated rats (B and C) . lycine an increase on treated rats .Argenine numerically increased on B and decreased on C .Hydroxyproline had been decreased on B group only and so for Proline on (B and C) when compared with group A (Control) . as shown in table 10,11 and 12. And figures 6,7 and 8.

Table10-Determination of serum amino acids composition : After three weeks of treatment group A (Control):

	Reten . Time {min}	Amount {umol\l}	Amount [%]	Compound Name
1	5.896	78.376	3.1	Taurin
2	8.579	1023.102	40.7	Urea
3	19.915	3.114	0.1	Aspatric acid
4	26.472	78.101	3.1	Threonine
5	28.611	113.462	4.5	Serine
6	31.637	14.191	0.6	Asparigine
7	33.499	46.396	1.8	Glutamic acid
8	46.653	146.165	5.8	Glycine
9	48.787	266.241	10.6	Alanine
10	50.691	23.377	0.9	Citruline
11	52.904	2.317	0.1	a-Aminobutyric Acid
12	56.323	47.762	1.9	Valine
13	58.925	3.369	0.1	Cystine
14	61.699	14.754	0.6	Methionine
15	64.829	33.293	1.3	Isoleucine
16	66.459	57.122	2.3	Leucine
17	70.123	8.290	0.3	Tyrosine
18	73.275	24.032	1.0	Phenylealanine
19	75.080	4.219	0.2	Homocystine;
20	76.048	13.217	0.5	Histidine
21	76.685	0.314	0.0	1-Methylhistdine
22	79.384	16.821	0.7	Tryptophane
23	80.259	47.282	1.9	Ornthine
24	81.741	66.027	2.6	Lysine
25	83.864	337.943	13.4	Ammonia
26	90.301	44.235	1.8	Argenine
	total	2513.520	100.0	

	Reten . Time {min}	Amount {umol\l}	Amount [%]	Compound Name

1	22.403	17.188	12.7	HYdroxy-prolline
2	43.648	117.666	87.3	Proline
	Total	134.855	100.0	

Figure 6: Determination of serum amino acids composition on normal rats group A after three weeks of treatments

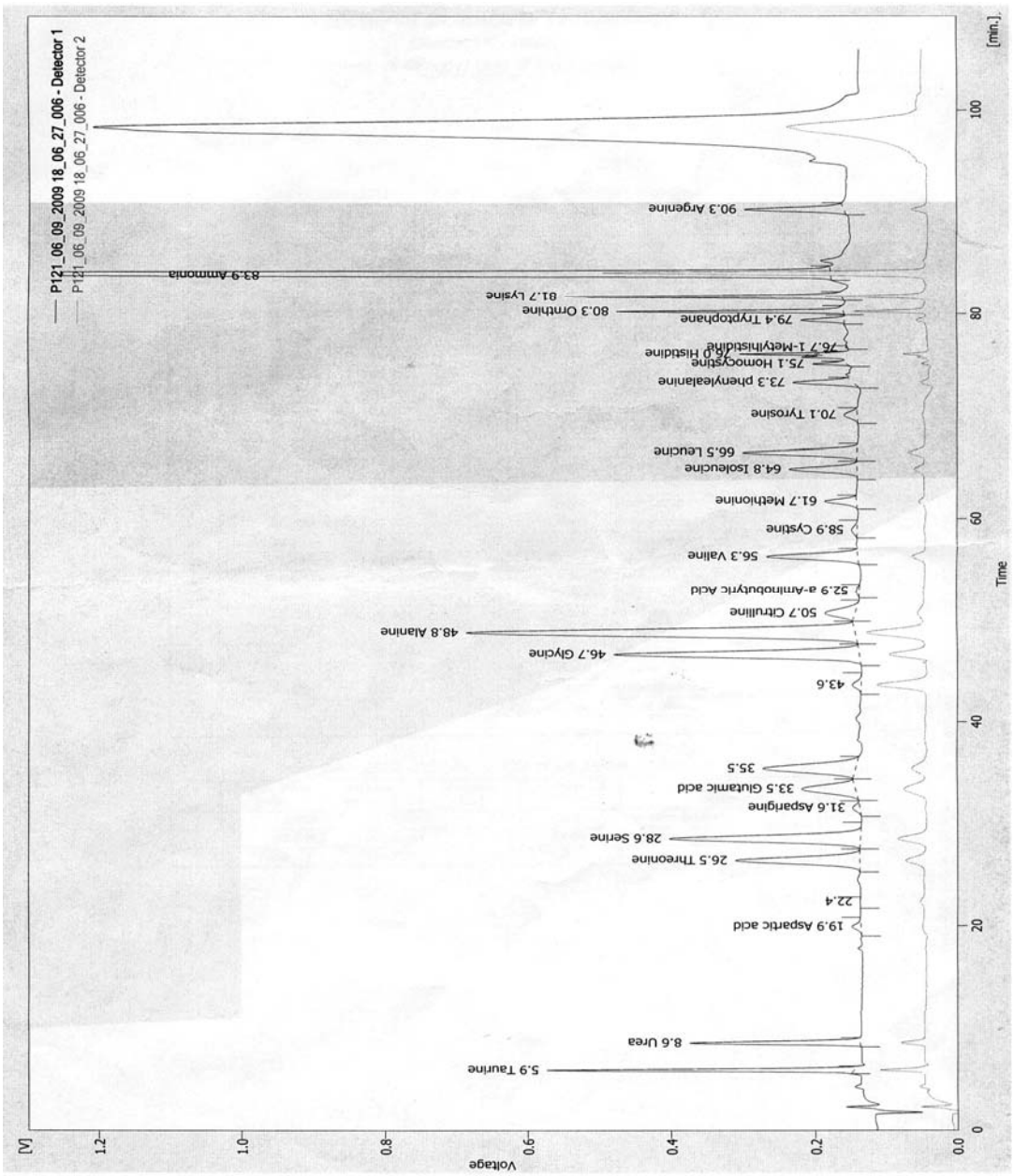


Table 11- Determination of serum amino acids composition : after three weeks of treatment group B:

	Reten . Time {min}	Amount {umol\l}	Amount [%]	Compound Name
1	5.899	77.913	3.1	Taurin
2	8.616	913.904	36.4	Urea
3	20.029	0.702	0.0	Aspatric acid
4	26.544	93.676	3.7	Threonine
5	28.683	108.685	4.3	Serine
6	31.717	16.262	0.6	Asparigine
7	33.693	47.137	1.9	Glutamic acid
8	46.675	165.958	6.6	Glycine
9	48.795	196.892	7.8	Alanine
10	50.856	12.874	0.5	Citruline
11	56.325	44.666	1.8	Valine
12	58.896	1.634	0.1	Cystine
13	61.731	14.196	0.6	Methionine
14	64.787	35.175	1.4	Isoleucine
15	66.437	71.365	2.8	Leucine
16	70.195	15.345	0.6	Tyrosine
17	73.397	30.535	1.2	Phenylealanine
18	75.112	4.462	0.2	Homocystine;
19	76.075	22.487	0.9	Histidine
20	76.720	0.585	0.0	1-Methylhistdine
21	79.467	17.102	0.7	Tryptophane
22	80.307	57.935	2.3	Ornthine
23	81.819	97.766	3.9	Lysine
24	83.877	410.219	16.3	Ammonia
25	90.520	53.069	2.1	Argenine
	total	2510.544	100.0	

	Reten . Time {min}	Amount {umol\l}	Amount [%]	Peak Type	Compound Name
1	22.528	14.901	15.0	Ordnr	HYdroxy-prolline
2	43.624	84.474	85.0	Ordnr	Proline
	Total	99.376	100.0		

Group B :Normal rats treated by *Boscia senegalensis* leaves water extract(200mg kg^{-1}).

Figure 7: Determination of serum amino acids composition on normal rats group B after Three weeks of treatments

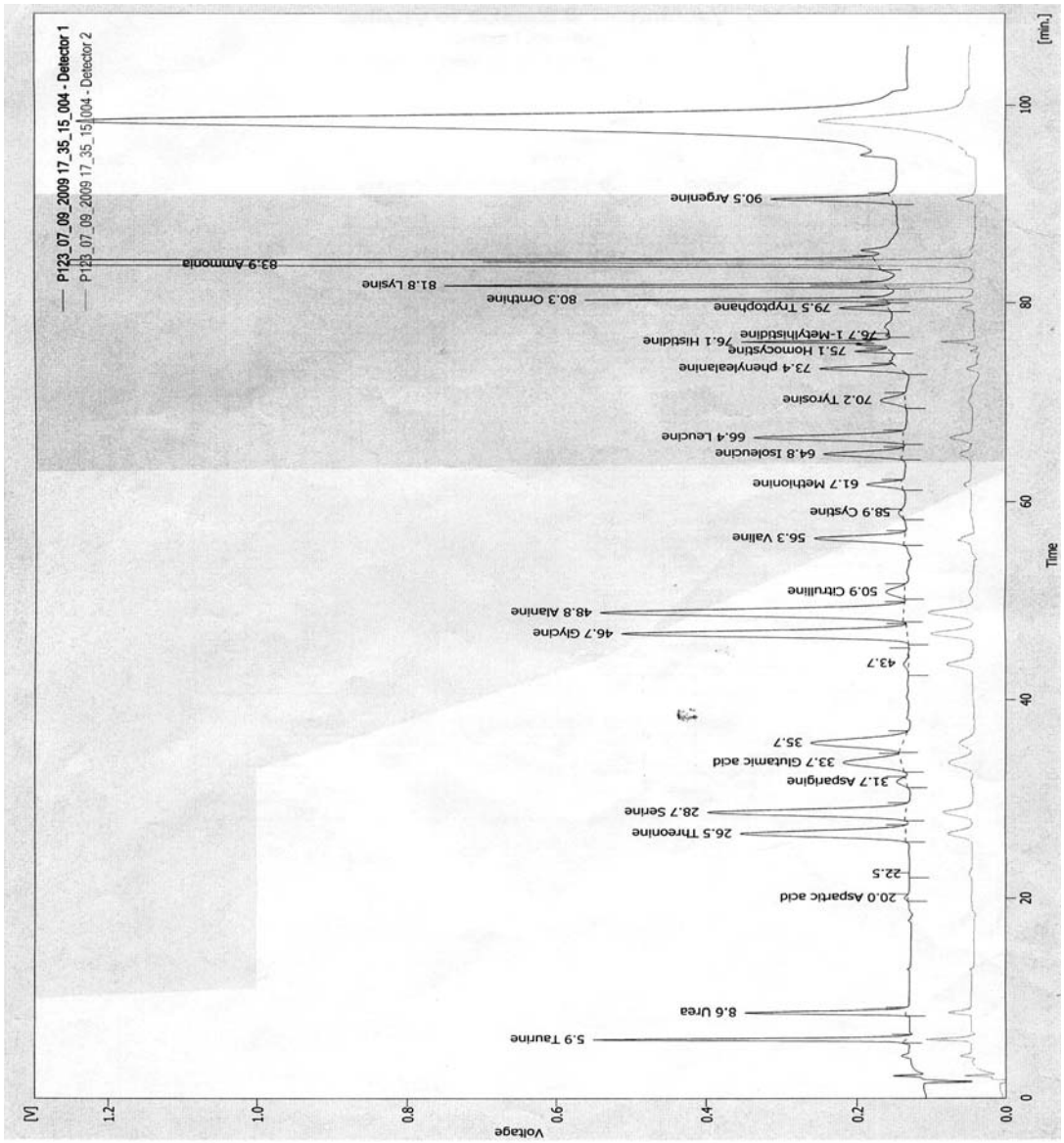


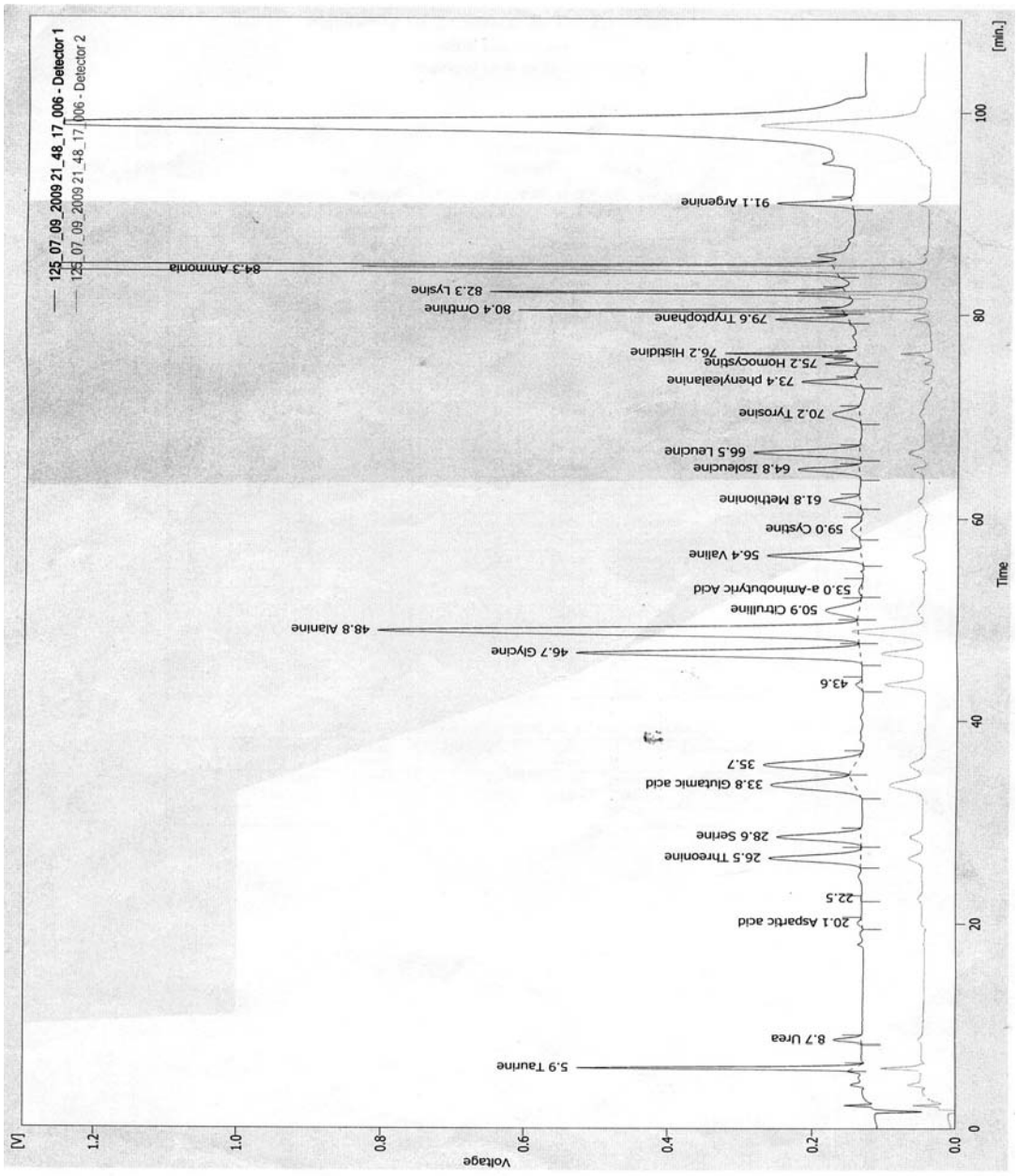
Table 12-Determination of serum amino acids composition: after three weeks of treatment group C:

	Reten . Time {min}	Amount {umol\l}	Amount [%]	Compound Name
1	5.909	70.965	3.8	Taurin
2	8.664	178.202	9.5	Urea
3	20.085	1.346	0.1	Aspatric acid
4	26.520	5.624	3.0	Threonine
5	28.619	47.82	2.6	Serine
6	33.757	74.048	4.0	Glutamic acid
7	46.693	169.303	9.0	Glycine
8	48.848	328.726	17.5	Alanine
9	50.909	26.717	1.4	Citruline
10	52.968	2.533	0.1	a-Aminobutyric
11	56.381	50.007	2.7	Valine
12	58.955	6.224	0.3	Cystine
13	61.800	14.992	0.8	Methionine
14	64.848	28.000	1.5	Isoleucine
15	66.464	52.876	2.8	Leucine
16	70.243	18.630	1.0	Tyrosine
17	73.429	23.443	1.3	Phenylealanine
18	75.176	4.592	0.2	Homocystine
19	76.176	22.615	1.2	Histidine
20	79.557	27.687	1.5	Tryptophane
21	80.408	65.288	3.5	Ornthine
22	82.291	102.816	5.5	Lysine
23	84.317	466.194	24.9	Ammonia
24	91.080	34.561	1.8	Argenine
	total	1873.208	100.0	

	Reten . Time {min}	Amount {umol\l}	Amount [%]	Peak Type	Compound Name
1	22.528	14.901	15.0	Ordnr	HYdroxy-prolline
2	43.624	84.474	85.0	Ordnr	Proline
	Total	99.376	100.0		

Group C:Normal rats treated by *B. senegalensis* leaves water extract(400mg/kg^{-1})

Figure 8: Determination of serum amino acids composition on normal rats group C after three weeks of treatments



3.5 The effects of oral administration of *Boscia senegalensis* leaves water extract on liver and kidney tissues:.

Histopathological result that: Glumerular alteration, necrosis, segmentaion, packing of glumerular tubule .fatty vaculation and hemorrhage(cortex) dilatation of medulla on kidney.

Liver: fatty cytoplasmic of the centrelobular hepatocytes ,necrosis and hemorrhage. As shown in figures 9, 10 and 11.

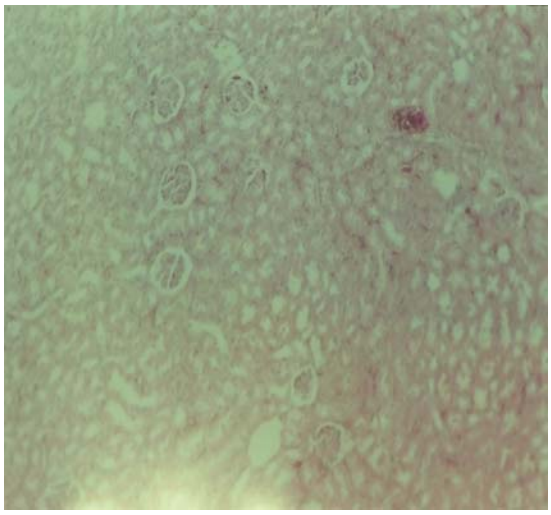


Figure 9: ***Kidney photomicrograph section of rat administered leaves Water extract of Boscia senegalensis (400mg kg¯1) showing Glumerula tubule alteration,necrosis,segmentation (H& E) X 200***

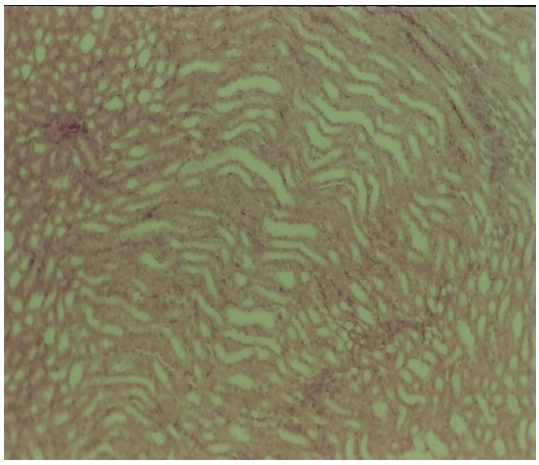


Figure10 ***: Kidney photomicrograph section of rat administered water extract of Boscia senegalensis (400mg kg^{-}1) showing dilatation of tubule (H& E) X 200***

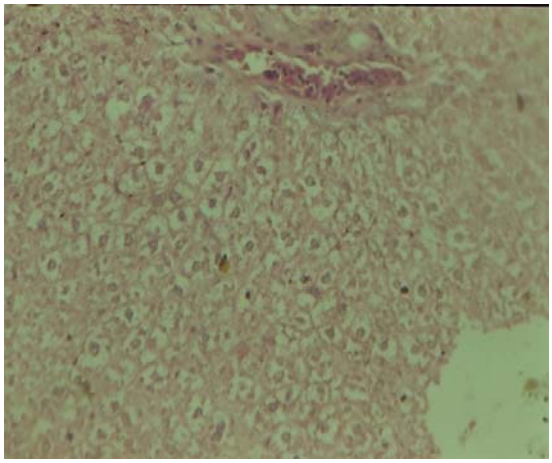


Figure 11: ***Liver photomicrograph section of rat dministered leaves extract of Boscia senegalensis (400 mg kg¯1) showing Cytoplasmic fatty vaculation centrilobular hepatocyte &necrosis hemorrhage on hepatocyte***

(H& E) X 200

Chapter four

Discussion

This study showed the effect of oral administration of ***Boscia* senegalensis** leaves water extract in two different concentrations on blood content (RBC, WBCs, HB , MCH, MCHC and platelets) and on serum ALT,AST , ALP , Urea, Creatinine, amino acid composition ,electrolyte sodium, potassium on Wister albino rats. In this study, the administration of water extract of ***Boscia senegalensis*** leaves in different concentration on blood content (RBCs , WBCs ,HB , MCH, MCH ,MCHC and platelets) had a significant effect on HB and RBCs there was an increase HB and decrease RBCs, when compared with Control. This deficiency on RBCs count and increase of hemoglobin concentration indicated that there were abnormal erythropoiesis this may appears as production of incompletely hemoglobinated erythrocyte or abnormal cells characterized both by deficient in number and having morphologic abnormalities**(Coles,1974).**

So this reduction on number of Red blood cell result may be attributed to hemolysis, which is the discharging of the hemoglobin from the Red cell so that it becomes in the plasma or other medium surrounding the cell, It is known that some substances like saponin lower surface tension produce hemolysis.It probably combine with some constituent of cell membrane or other wise alter it **(Duke, 1955).** So this reduction on number of Red blood cell and increased hemoglobin this indicate the hemolysis which may be attributed to saponin on water extract of leaves of ***Bsenegalensis.***

The liver enzymes, Aspartate and Alanine aminotransferases (AST and ALT) are involved in amino acid metabolism. Large amounts of AST are present in the liver, kidneys, cardiac muscle and skeletal muscle. However, ALT is known to be found principally in the liver **.**Serum ALT and AST levels were always found to increase in liver cell damage and the greater the degree of liver damage the higher the activities of both enzymes(**Cheesbrough,1991).**

Alkaline Phosphatase (ALP) is a marker enzyme for the plasma membrane and endoplasmic reticulum. It is often used to assess the integrity of plasma membrane **(Akanji et al., 1993).** It is also related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis of the enzyme in presence of increasing biliary pressure. Significant elevation of serum alkaline phosphatase is an indication of cholestasis with no effective control of ALP activity towards improvement in the secretory function of the hepatic cell **(Van Hoof and De Broe, 1994)** .

In this study from the result obtained water extract of ***Boscia senegalensis*** had no significant change on serum AST, ALT, ALP. This indicated that there were no obvious pathological changes occurs on liver, kidney or muscles **(Coles, 1974).** Thus there were slight increase on AST level which may be associated with cellular necrosis on liver or on different tissues (skeletal, cardiac muscle and liver). By contrast elevation of ALT level indicates the cellular degeneration or destructions on liver **(Coles, 1974).** These could be attributable to the effect of the extract.

Neither serum urea concentration nor Creatinine in the treated rats showed significant changes when compared with the control group, but there is numerical decreased on group C this alteration on urea level affected not only by alteration in renal function but may alter by certain physiological factor or disease not primarily

of renal origin **(Coles, 1974).**Also the slight decrease in Creatinine may indicate to alter on glomerular filtration or reabsorbed tubule **(Coles, 1974).**

The histopathological changes observed in the liver and kidneys of rats administered higher doses of aqueous extract of ***B. senegalensis*** caused the alterations of the hepatorenal indices. These could be attributable to the effect of the extract. This may not be a surprise because liver and kidney are sites for xenobiotic metabolism and excretion, respectively. It is known that various anti nutritional substances and xenobiotic chemicals like saponins and tannins cause haemolysis, nutrient malabsorption and abnormal haemopoesis which could arise from kidney and liver damage **(Chubb, 1982; Connig, 1993).**

Also some alkaloids have cytotoxic effect on organs; they damage the cells of the liver, lungs heart and kidney **(Harborne, 1972).** The toxicity of the leaves extract of ***B. senegalensis*** may be consequent upon the combined toxicity of constituents detected in the extract such as alkaloids and saponins.

From all previous findings the result is ***Boscia senegalensis*** leaves water extract on dose 400mg/kg has toxic effect on Wister albino rats. This result nearly agree with **(Nongoniermsierm ,2007)** whose said the extracts of the leaves of ***B .senegalensis*** at dose of 1 g/kg were very toxic,The LD50 was equal to 0, 44 g/k.

Conclusions and Recommendations

Conclusions :

From observations and results of this investigations, it can be concluded that:

1. The administration of ***Boscia senegalensis*** water extract to normal rats had a significant effect on Red blood cell count 7.598±02153* and hemoglobin concentration . Decreased Red blood cell count and increased hemoglobin concentration 15.35±0.395*.

2. The administration of ***Boscia senegalensis*** water extract to normal rats had no significant effect on serum enzyme ALT, AST and ALP concentration or on serum urea and Creatinine on treated group.But ALT and AST had been numerically increased ,by contrast Urea and Creatinine had been decrease (after two weeks from the start of experiment).

   After three weeks there were numerically decreased ALT, AST, ALP and urea concentration on group B in contrast group C had increased ALT, AST, ALP.

3. The administration of ***Boscia senegalensis*** extract to normal rats had no significant effect on serum electrolyte sodium and potassium. But there were numerically increased on sodium and potassium after two weeks of the experiment on B and C, but there were numerically increased on sodium and decreased potassium after three weeks.

4-The administration of ***Boscia senegalensis*** wate*r* extract to normal rats had an effect on liver and kidney organ of group C (4oomg/kg).

RECOMMENDATIONS

Because *Boscia senegalensis* has different uses in folkloric medicine and nutrition for human and animal I recommended that:

1. More pharmacological studies on this plant to determine the active principle of *Boscia senegalensis*.

2. More pharmacological studies on this plant to determine lethal dose.

3. More research should be carried out to determine whole biological effect.

4. More research should be carried out to determine the carcinogenic effect

References

Abdelmuti,O.M. (1991). *Biochemical and Nutritional Evaluation of Famine Foods in Sudan*. Ph.D. Thesis, University of Khartoum.

Ahmed , O.M.M. (1988) . *Toxicological studies on fruit of Balanites aegyptiaca* (Hijlij tree). Ph.D .Thesis , University of Khartoum. Sudan.

Anderews,F.W. (1950) .*The flowering plant of the Anglo Egyptian Sudan*, Pup.T.BUNCLE and CO.LTD ARBR0ATA.Scotland.

Asima , C . and Pakrashi, S.C. (1995) .*The treatise on Indian medicinal plants* .Vol.4. **P**: 325.

Akanji, M.A., Olagoke, O.A.and Oloyede, O.B. (1993). Effect of chronic con sumption of metabi-sulphite on the integrity of the kidney cellular system. Toxicology,**81**:173-179.

Almagboul,A .Z, Bashir.AK .,Karim.A, Salih.M,Farouk.A, Khalid,S.A. (1988) Antimicrobial activity of certain Sudanese plants used in folkloric med-cine.Screening antifungal activity.Fitoterabia.(**59) :** 393_396 .source was an original research paper .

Bakhiet, A.O. (1995). *Comparative effects on chicks of some indigenous plants* .Ph.D. Thesis, University of Khartoum, Sudan.

Baoua,M.,Fayn,J. and Bessiere. J.M.(1976). Essais Phytochemique preliminatary on some medicinal plant in Niger .*plant Med*.**10** :251-266.

Bohn JA, BeMiller JN. (1995). (1 3)-ß-D-glucans as biological response modifiers: A review of structure-functional activity relationships. Carbohydr. Polym. 28: 3-14.

Bullough.C.W,Leary.W.P,1982) Trop.Geograph.Med.**34**:810

www.nlm.nih.gov/hmd/breath

Burck.P.J.,Thakkar,A.L.,Zimmermann.R.E.(1982). Antifertility action of a sterol sulphate in rabit.*Jou Reproduction and fertility* .**66**.109:112

www.nlm.nih.gov/hmd/breath

Cheesbrough, M., (1991). *Medical Laboratory Manual for Tropical Countries*. **2nd** Edn., University Press, Cambridge.P: 508-511.

Chi,H.J. , Choi,J.R.Yu.S.C. (1985) .Pharmacological studies on (Ho_jang).Phytochemical study of the rhizome of *poly gonum* elliprum Miso ko-rean.*Jou.Pharmmacognsy*. **13.** P: 145_152.

Chubb, L.G.(1982). *In Recent Advances in Animal Nutrition.* W. Harvesign But-terworths, London, p: 21-37

Coles,E.H .(1974) .*Veterinary Clinical Pathology* .**2nd** edition. Pub.W.B Saunder Company USA.

Connig, D.M.(1993). *Experimental toxicology*. The Basic Issues. Anderson D. and D.M. Conning (Eds.), **2nd** Edn. P: 1-3.

Delaveau ,P.,Koudogbo,B.and Pousset.(1973). Alkaloid from the Capparidaceae. *Jou Phytochemistry* .**12**. P: 2893-2895.

DFID: **The British Department for International Development ,The European Union and the World Agroforestry Centre. (2009)** Agroforestree database

. http://www.worldagroforestry.org/af/treedb/index.php?keyword=Poison

Dicko,MH. ,Marjo S., leeuwen,V., Alfred S., Hilhorst, T. R and Beldman,G.(2001). Polysaccharide hydrolases from leaves of *Boscia senegalensis* Properties of endo-(1→3)-β-d-glucanase . *Jour Applied Biochemistry and Biotechnology*, Publisher Humana Press Inc. **94**: 225-241

Drury, R.A. and Wallington, E.A. (1980). Carleton's Histological Techniques. **5th** ed., Oxford, New York, Toronto.

http://www.worldagroforestry.org/af/treedb/index.php?keyword=Poison

Duke,H.H,D.V.M.,MS. (1955).The physiology of domestic animals.**7th** edn .vol 1020 . P: 26_28.

ELGaddal, J.A. (1993). Thesis. *The use of plant coagulants for water treatment in rural Sudan,* University of Surrey .

Elgazali. G. E.B, Eltohami .M.S.,, Elegami.A.B.,Abdalla .W.S., Mohammed .M.G.(1997).*Medicinal plant of Northern Kordofan* .National Center for Researrch(MAPRI) Khartoum. Sudan .Part5

Elgazali, G.E.B., Eltohami .M.S., Elegami.A.B.,Abdalla .W.S., Mohammed .M.G.,(1987).*Medicinal plant of Sudan part II.* National Center for Researrch(MAPRI) Khartoum.

Elgazali, G.E.B., Eltohami .M.S., Elegami,A.B.(1994). *Medicinal plant of Sudan part III (Medicinal plant of The White Nile province).* National Center for Researrch(MAPRI) Khartoum .

ELkhier , Y.M.,Salih,MH. (1980) Investigation of certain plant used in Sudanese folk Medicine. *Fitoterapia*.143-147. source was an original research paper .University of Khartoum faculty of pharmacy Khartoum Sudan

Gupta ,M.B.,North,R.,Srivastava,N.,Shanker,K.,Kishorbhargava,KP.(1980).

Antiinflamotory and Antipyretic activity of B sisterol, Plant.Medica., **39**:157-163.

Grindely,D.N. (1948) . Jou soc,chem. Ind 67_230

Harborne, J.B. (I972). *Phytochemical Ecology*. **8th** Edn., Academic Press Inc., London,NewYork . P :182-195..

Harborne , J. B (1984). *Phytochemical methods* . **2nd** edition.

Kerharo, J. ,Adams, J.R. (1974). *La pharmacopée sénégalaise*

traditionnelle, plantes médicinales et toxiques (Vigot Frères eds.),Paris, France

Kim, T. R., Pastuszyn, A. (1997). The nutritional composition of seeds from *Boscia senegalensis* (Dilo) from the Republic of Niger. *Jour of Food Composition and Analysis*. **10**(1): 73-81. {A}

Killian , C. **(1937)** . *Contribution à l'étude écologique des végétaux du Sahara et du Soudan tropical*. Bull. Soc. Hist. Nat. Afrique du Nord **28**:12-18.

www.nap.edu/openbook.php?record_id=11879&page...

Kjaer, A .,Schuster,A.,Delaveau, P., koudogbo ,B. (1973).Glucosinolate in *Boscia senegalensis* Phytochemis**12**:725-726. Source was an original research paper

Laurens ,A .(1985). Pharmazie 40(7):482

Longoay,G ., Marlier,M .,Seck,D., Haubruge ,E.,Wathelet,J.P.,Coulibally A.D., Gaspar ,C.and Severin,M. (1994). Bull.Res.agro.Gembloux,Belgium.:117-124.

Malani,T.,Vanithakumari,G.(1991) .The anti fertility effect of in male albino rats. *Jour Ethno pharmacology*. **(35)** :14 7_153.

Maurice ,I.W.U.(1993).*Hand book of tropical plant of Africa*. vol 435. p :132-133. CRC press , printed in USA.

www4.fao.org/cgibin/faobib.exe?rec_id=326840&database=faobib&search_type=link&table=mona&back_path=/faobib/mona&lan...

Mendehall, W. S. (1971). *Introduction of Probability Statistics***. 3rd ed.**, Wadswarch Publishing Co., Belmont, California, USA.

Monica, C. (1992). *Medical Laboratory Manual for Tropical Countries*. **2 nd** ed., pp 465-472, Butterworth-Heinemann Ltd, UK.

NRC: National Research Concil . (2008). Aizen-Mukheit *Lost crop of Africa Fruit.part III* . 220_230. National Academies Press.Washington DC.

www.nap.edu/openbook.php.

Neuwinger ,H.D . (1996). *African ethanobotany:poison and drug chemistry, pharmacology ,toxicology*. Vol 941.p:327-329.Pub :CRC press.

www.nlm.nih.gov/hmd/breath

Nongoniermsierm, R.M.,Ndiaye .A., Ndiaye M., Faye B.,(2007) .

Anti-inflammatory activity of the aqueous and alcoholic leaves extract of Boscia senegalensis (pers) lam. ex. poir. capparidaceae *Jour Le pharmacien d'Afrique n*°.,**199** : 9-15

Omer, S.A., Ibrahim, F.H., Khalid, S.A. and Adam, S.E.I.(1992). Toxicological interaction of *Abrus precatorius* and *Cassia Senna* in the diet .

Pakarashi, A ., Basak ,B. (1976) . Abortifacient effect of steroid from anannas cosmosus and their analogues in mice.*Jou;Reproduction and fertility*.**46:** 461 _462.

Pauli , N., Sequin .U ., Walter . A . (1990). Helr.Chim. Acta 73 (3) 578

www.nlm.nih.gov/hmd/breath

Ryals,J.,Neuenschwander,U.,Willits,M.,Molina,A.,Steiner,H.Y.and Hunt,M.(1996). *Plant cell.* **8th edn**. P: 1809-1819.

Salih, O.M., Nour, A.M. (1991). Chemical and nutritional composition of two famine food sources used in Sudan, Mukheit (Boscia senegalensis) and Maikah (Dobera roxburghi). *Jour of the Science of Food and Agriculture*. **57**(3): 367-378.

Seck, D., Lognay, G.,Haubruge, E.,Marlier, M. and Gaspaur,C.(1995). Alternative protection of cowpea seeds against Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) using hermetic storage alone or in combination with *Boscia senegalensis* (Pers.) Lam ex Poir .*Jour of Stored Products Research*., **32:**39-44.

Seck, D., Lognay, G. (1993). *Biological activity of the shrub Boscia senegalensis* (Pers.) Lam. ex Poir. (Capparaceae) on stored grain insects. *Jour of Chemical Ecology* **19**(2): 377-389 .

Van Hoof, V.O. and De Broe, M.E. (1994) Interpretation and clinical significance of Alkaline phosphatase isoenzyme patterns. Crit. Rev. Clin. Lab. Sci., 31:197-293.

Varley, H. (1967). The determination of Na and K by flame photo. *In: Pract. Clin. Biochem*., **4th** edn., Heineman W. Med. Books Ltd, Int. Book Inc., New York.

Walter , A ., Sequin , U . (1990) . Phytochemistry 29:2561

www.nlm.nih.gov/hmd/breath